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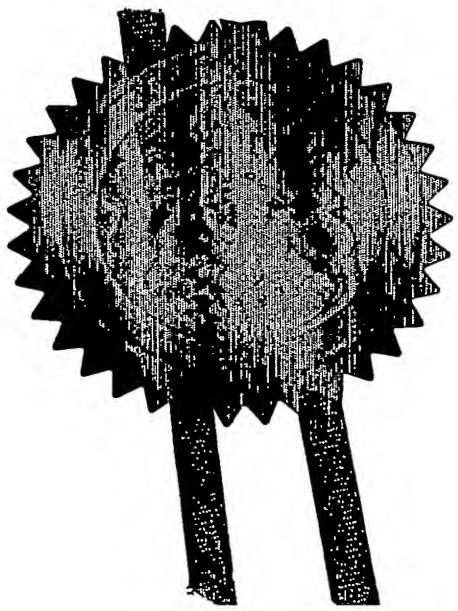

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2. Patent application number *(The Patent Office will fill in this part)* — 0308734.3

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — AXCESS LIMITED
44 Esplanade, St Helier
Jersey
Channel Islands JE1 3UQ

Patents ADP number *(if you know it)* — 8538528002

If the applicant is a corporate body, give the country/state of its incorporation — British body corporate

4. Title of the invention — UPTAKE OF MACROMOLECULES

5. Name of your agent *(if you have one)* — J.A. KEMP & CO.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — 14 South Square
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London
WC1R 5JJ

Patents ADP number *(if you know it)* — 26001

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Signature J.A. KEMP & CO. Date 15 April 2003

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G W McCluskie
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UPTAKE OF MACROMOLECULES

This invention relates to a pharmaceutical composition and the use of materials having efficacy in enhancement of uptake of macromolecules from the lumen of the intestine across the gut wall and into the rest of the body in the preparation of a medicament. In particular, formulations are described in which bile salts are combined with other materials in such a way as to improve the performance of these bile salts (in terms of efficacy, reproducibility, or stability) compared with formulations employed to date.

Bile salts are already recognised as highly effective permeation enhancers in the intestine. They are surface-active compounds that can incorporate readily into phospholipid membranes of cells and bring about a number of changes in cell behaviour and morphology. While the precise mechanism of action is unknown, one possible mode of action is the transient opening of tight junctions in between enterocytes, allowing materials in the vicinity to pass in between these cells and into the extracellular fluid basal to the enterocyte layer. Evidence for this is provided by experiments from laboratories demonstrating reduction in trans epithelial electrical resistance (TEER) of enterocyte cell monolayers, indicative of the formation of aqueous channels in between cells, capable of conducting electricity unimpeded.

Since peristaltic action in the intestine tends to encourage rapid dispersion of pharmaceutical formulations over a larger surface area, it is important that the bile salts in the formulation should dissolve rapidly, and remain in the form of a solution for a long as possible, so that the local concentration of bile salt may be maintained as high as possible in order for it to exert its maximal effect. For this reason, most workers have employed conjugated bile salts such as taurocholate or taurodeoxycholate, since these are readily soluble over a wide range of pH, including pH values as low as 3, which may be found in the intestine. These bile salts, however, are not as efficacious as some non-conjugated, particularly deoxy bile salts, such as chenodeoxycholate or deoxycholate, although these are often not soluble at pH values much below neutrality. Thus the performance of non-conjugated bile salts as a permeation enhancer would be expected to be very variable, depending upon the pH in the area of the intestine where it was operating, which in turn would determine

whether the bile salt was present as a solution (efficacious) or a precipitate (poorly efficacious).

In order to overcome this lack of reproducibility, approaches have been described in which the bile salt is combined with an agent which adjusts the pH in the gut to greater than 7.5, in order to ensure that the bile salt is in soluble form. Unfortunately, the inclusion of such agents in the formulation can adversely affect the integrity and stability of sensitive actives included in the formulation such as peptides and proteins. Also, the pH optimum for intestinal proteases is known to be around pH 8, so that the adoption of such an approach may increase degradation due to endogenous protease activity.

The present invention describes an alternative approach in which bile salts are combined with additives that, surprisingly, are able to maintain these bile salts in solution at pH values below 7, equivalent to those pH values commonly encountered in the small intestine. In particular, it has been found that co-formulation of bile salts either with propyl gallate or derivatives, or with salts of organic acids, can give rise to solid powders which, when added to simulated intestinal fluids in a wide range of different proportions, are readily dissolved, even when the initial pH of the fluid is as low as pH5. Suitable simulated intestinal fluids which may be used as models for the lumenal contents of the intestine in either fed or fasted states are described by Dressman and coworkers (Dressman, B Jennifer & Reppas, Christos. *European Journal of Pharmaeuetical Sciences*. **11** Suppl 2 (2000) S73-S80 "*In vitro-in vivo* correlations for lipophilic, poorly water-soluble drugs.").

The compositions described in this invention are particularly advantageous when preparing solid-dose formulations for oral administration either as enteric-coated capsules or tablets. The solid components of the composition may also be incorporated into a pharmaceutical formulation as a dispersion in a non-aqueous liquid.

In the case of propyl gallate, which is a very water-insoluble material, it has been found, surprisingly, that co-formulation with bile salts, including chenodeoxycholate, can markedly enhance the solubilisation of propyl gallate in water, and particularly in simulated intestinal fluids. Thus, chenodeoxycholate and propyl gallate are able mutually to enhance each other's aqueous solubility at pH

levels below pH 7. In view of the fact that propyl gallate itself is highly water-insoluble, it would not be anticipated that addition to chenodeoxycholate would result in a formulation which was more readily soluble, and a person skilled in the art would not be motivated to attempt such a procedure.

The invention provides a pharmaceutical composition comprising a mixture of:

(a) an active macromolecular principle; and

(b) a non-conjugated bile acid or salt; and

(c) an additive chosen from propyl gallate and butyl hydroxy anisole and analogues and derivatives thereof, and an organic acid or a pharmaceutically acceptable salt thereof, or mixtures thereof, which additive is capable of allowing the non-conjugated bile salt to remain in solution when added to intestinal fluids at pH levels between 5 and 6.5, and which, when introduced into the intestine does not raise the pH of the intestinal fluid above pH 7.5.

The invention also provides the use, in a pharmaceutical composition, of a non-conjugated bile acid or salt, together with an additive chosen from propyl gallate and BHA and analogues and derivatives thereof, and an organic acid or a pharmaceutically acceptable salt thereof, or mixtures thereof as an enhancer for the absorption of macromolecules across the intestinal wall.

In a further embodiment the invention provides the use of a non-conjugated bile acid or salt, together with an additive chosen from propyl gallate and BHA and analogues and derivatives thereof, and an organic acid or a pharmaceutically acceptable salt thereof or mixtures thereof in the manufacture of a medicament containing an active macromolecular principle, in order to enhance absorption of the active macromolecular principle into the human or animal body.

The active macromolecular principles falling within the scope of the invention include all molecules capable of having a beneficial effect when absorbed into the human or animal body, especially through the intestinal wall. The beneficial effect may be, for example, therapeutic, cosmetic or preventative such as prophylactic or contraceptive. The active macromolecular principles can be of natural (biological), synthetic or semi-synthetic origin.

Macromolecules are preferably defined as molecules having a molecular weight of over 1000 Da, preferably over 2000 Da and most preferably over 3000 Da. Examples of macromolecules, including macromolecular active macromolecular principles, include:

1. Polypeptides and proteins such as insulin; calcitonin; human serum albumin; growth hormone; growth hormone releasing factors; galanin; parathyroid hormone; blood clotting proteins such as kinogen, prothombin, fibrinogen, Factor VII, Factor VIII of Factor IX; erythropoeitins and EPO mimetics; colony stimulating factors including GCSF and GMCSF; platelet-derived growth factors; epidermal growth factors; fibroblast growth factors; transforming growth factors; GLP-1; GAG; cytokines; insulin-like growth factors; bone- and cartilage-inducing factors; neurotrophic factors; interleukins including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; interferons including interferon gamma, interferon β-1a, interferon alphas; TNF alpha; TNF beta; TGF-beta; cholera toxin A and B fragments; E. coli enterotoxin A and B fragments; secretin; enzymes including histone deacetylase, superoxide dismutase, catalase, adenosine deaminase, thymidine kinase, cytosine deaminase, proteases, lipases, carbohydrases, nucleotidases, polymerases, kinases and phosphatases; transport or binding proteins especially those which bind and/or transport a vitamin, metal ion, amino acid or lipid or lipoprotein such as cholesterol ester transfer protein, phospholipid transfer protein, HDL binding protein; connective tissue proteins such as a collagen, elastin or fibronectin; a muscle protein such as actin, myosin, dystrophin, or mini-dystrophin; a neuronal, liver, cardiac, or adipocyte protein; a cytotoxic protein; a cytochrome; a protein which is able to cause replication, growth or differentiation of cells; a signalling molecule such as an intra-cellular signalling protein or an extracellular signalling protein (eg hormone); trophic factors such as BDNF, CNTF,NGF, IGF, GMF, aFGF, bFGF, VEGF, NT3, T3 and HARP; apolipoproteins; antibody molecules; receptors in soluble form such as T-cell receptors and receptors for cytokines, interferons or chemokines; proteins or peptides containing antigenic epitopes and fragments; and derivatives, conjugates and sequence variants of any of the above. These and other proteins may be derived from human, plant, animal, bacterial or fungal sources, and extracted either from natural sources, prepared as recombinants by fermentation or chemically synthesised.

2. Polynucleotides such as long-chain linear or circular single-, double- or triple-stranded DNA, single-, double- or triple-stranded RNA, oligonucleotides such as antisense DNA or RNA, and analogues thereof including PNA and phosphothioate derivates. In one embodiment it is preferred that the polynucleotides used in the invention contain a CpG motif. The coding sequence of the polynucleotide may encode a therapeutic product, in particular the coding sequence may encode an extracellular protein (e.g. a secreted protein); an intracellular protein

(e.g. cytosolic, nuclear or membrane protein); a protein present in the cell membrane; a blood protein, such as a clotting protein (e.g. kinogen, prothrombin, fibrinogen factor VII, factor VIII or factor IX); an enzyme, such as a catabolic, anabolic gastro-intestinal, metabolic (e.g. glycolysis or Krebs cycle), or a cell signalling enzyme, an enzyme which breaks down or modifies lipids, fatty acids, glycogen, amino acids, proteins, nucleotides, polynucleotides (e.g. DNA or RNA) or carbohydrate (e.g. protease, lipase or carbohydrase), or a protein modifying enzyme, such as an enzyme that adds or takes chemical moieties from a protein (e.g. a kinase or phosphatase); a transport or binding protein (e.g. which binds and/or transports a vitamin, metal ion, amino acid or lipid, such as cholesterol ester transfer protein, phospholipid transfer protein or an HDL binding protein); a connective tissue protein (e.g. a collagen, elastin or fibronectin); a muscle protein (e.g. actin, myosin, dystrophin or mini-dystrophin); a neuronal, liver, cardiac or adipocyte protein; a cytotoxic protein; a cytochrome; a protein which is able to cause the replication, growth or differentiation of cells; a protein which aids transcription or translation of a gene or regulates transcription or translation (e.g. a transcription factor or a protein that binds a transcription factor or polymerase); a signalling molecule, such as an intracellular or extracellular signalling molecule (e.g. a hormone); an immune system protein such as an antibody, T cell receptor, MHC molecule, cytokine (e.g IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, TNF-α, TNF-β, TGF-β), an interferon (e.g. IFN-α, IFN-β, IFN-γ), chemokine (e.g. MIP-1α, MIP-1β, RANTES), an immune receptor (e.g. a receptor for a cytokine, interferon or chemokine, such as a receptor for any of the above-mentioned cytokines, interferons or chemokines) or a cell surface marker (e.g. macrophage, T cell, B cell, NK cell or dendritic cell surfacemarker)(eg. CD 1, 2, 3, 4, 5, 6, 7, 8, 16, 18, 19, 28, 40, or 45; or a natural ligand thereof), a trophic factor (e.g. BDNF, CNTF, NGF, IGF, GMF, aFGF, bFGF, VEGF, NT3, T5, HARP) or an apolipoprotein; a tumour suppressor (e.g. p53, Rb, Rap1A, DCC or k-rev); a suicide protein (thymidine kinase or cytosine deaminase); or a gene repressor. The proteins and peptides encoded by the polynucleotides useful in the invention may be immunogenic i.e. contain an antigen specific to the activity of the protein against which antibodies are generated by the immune system.

The polynucleotide may have control sequences operably linked to the coding sequence. The control sequences may typically be those of any eukaryote or of a virus which infects such eukaryotes. The polynucleotide may comprise an origin of replication.

The polynucleotides may be chemically modified. This may enhance their resistance to nucleases or may enhance their ability to enter cells. For example, phosphorothioate oligonucleotides may be used. Other deoxynucleotide analogs include methylphosphonates, phosphoramidates, phosphorodithioates, N3'P5'-phosphoramidates and oligoribonucleotide phosphorothioates and their 2'-O-alkyl analogs and 2'-O-methylribonucleotide methylphosphonates. Alternatively mixed backbone oligonucleotides (MBOs) may be used. MBOs contain segments of phosphothioate oligodeoxynucleotides and appropriately placed segments of modified oligodeoxy- or oligoribonucleotides. MBOs have segments of phosphorothioate linkages and other segments of other modified oligonucleotides, such as methylphosphonate, which is non-ionic, and very resistant to nucleases or 2'-O-alkyloligoribonucleotides.

The polynucleotide suitable for use in the invention is preferably in a form in which it is substantially free of or associated with cells or with cellular, prokaryotic, eukaryotic, nuclear, chromatin, histone or protein material. It may be in substantially isolated form, or it may be in substantially purified form, in which case it will generally comprise more than 90%, e.g. (more than or at least) 95%, 98% or 99% of the polynucleotide or dry mass in the preparation. Thus the polynucleotide may be in the form of 'naked DNA'.

3. Polysaccharides such as heparin, low-molecular weight heparin, polymannose, cyclodextrins and lipopolysaccharide.

4. Any or all of the above either separately or in combination with each other (for example in the form of a heteroconjugate), or with additional agents.

In preferred embodiments of the invention, the active macromolecular principle to be absorbed is selected from calcitonins, insulin, low molecular weight heparin, erythropoeitin, human growth hormone and parathyroid hormone.

Most preferably, the macromolecular principle is insulin, and then, the composition suitably further comprises an insulin sensitising agent. Insulin sensitisers are capable of increasing the body's response to the insulin absorbed. Suitable sensitisers are known in the art and examples include troglitazone, pioglitazone, rosiglitazone and other members of the glitazone class of molecules.

The non-conjugated bile acid or salt is suitable chosen from chenodeoxycholic acid, deoxycholic acid, cholic acid or the sodium salts thereof. The bile salt is preferably chenodeoxycholate.

The additive is chosen from propyl gallate and butyl hydroxy anisole and analogues and derivatives thereof, and an organic acid or a pharmaceutically acceptable salt thereof, or mixtures thereof, which additive is capable of allowing the non-conjugated bile salt to remain in solution when added to intestinal fluids at pH levels between 5 and 6.5, and which, when introduced into the intestine does not raise the pH of the intestinal fluid above pH 7.5.

The additive may be propyl gallate or a derivative thereof. Suitable derivatives of propyl gallate include esters of gallic acid. The esters may be linear or branched chain C_{1-12} alkyl, C_{1-12} alkyloxy, C_{1-12} alkylthio or C_{2-12} alkenyl esters. The compounds are optionally substituted with halogen, linear or branched chain C_{1-12} alkyl, C_{1-12} alkyloxy, C_{1-12} alkylthio or C_{2-12} alkenyl esters.

The additive may also be BHA or an analogue or derivative thereof. Suitable analogues or derivatives thereof include analogues and derivatives of hydroxy anisole where the methyl group or the methoxy group linked to the aromatic ring and/or the hydrogen ortho to the hydroxyl group are replaced by linear or branched chain C_{1-12} alkyl, C_{1-12} alkyloxy, C_{1-12} alkylthio or C_{2-12} alkenyl, either unsubstituted or substituted in any position, especially by halogen atoms.

Suitable organic acids that may be employed as additives in the invention include straight chain or branched, saturated or unsaturated long chain fatty acids (C_4 and above) such as oleic acid and derivatives, such as chloro or methyl derivatives, or straight chain or branched, saturated or unsaturated medium chain fatty acids (preferably C_{6-14}) such as capric, caproic, caprylic and lauric acids and their derivatives. The pharmaceutically acceptable salt of the organic acid is generally an alkali metal salt, preferably the sodium salt. The sodium salts are preferred additives.

The ratio of bile salt to additive, e.g. propyl gallate, is preferably from 1:1 to 5:1, more preferably from 4:3 to 5:2, and most preferably about 2:1 by weight.

The amount of non-conjugated bile salt/additive (absorption enhancer) and active macromolecular principle in the composition of the invention is chosen so as to achieve, at the intestinal cell barrier layer (intestinal wall), an effective concentration of non-conjugated bile salt/additive absorption enhancer so as to cause enhanced absorption in the co-presence of a suitable amount of the active macromolecular principle which, when absorbed, will exert its normal beneficial effect. The practitioner of the invention would select the amounts of the non-conjugated bile salt/additive absorption enhancer and active macromolecular principle on the basis of the amount (for example, blood concentration level) of the

active macromolecular principle concerned which is necessary for therapeutic effectivity. For example, the weight ratio of the total weight of the non-conjugated bile salt/additive absorption enhancer to the active macromolecular principle in the mixture contained in the capsule may preferably be from1:1 to 200:1, more preferably from 3:1 to 100:1, and most preferably from 5:1 to 50:1.

The absolute amount of the active macromolecular principle would be selected on the basis of the dosage of the substance required to exert the normal beneficial effect with respect to the dosage regimen used and the patient concerned. Determination of these dosage amounts falls within the mantle of the practitioner of the invention.

The composition of the invention may further comprise one or more other absorption enhancer compounds, for example, medium chain monoglycerides, chelating agents etc.

The composition of the invention may optionally further comprise any conventional additive used in the formulation of pharmaceutical products including, for example, anti-oxidants, anti-microbials, suspending agents, fillers, diluents, disintegrants, swelling agents, viscosity regulators, plasticisers and acidity regulators (particularly those adjusting the intestinal milieu to between 7 and 7.5).

In the composition of the invention where the mixture is contained in a capsule or tablet, the formulation is preferably substantially anhydrous. In more preferred embodiments of the invention the entire composition is substantially anhydrous. Substantially anhydrous in the context of this invention means less than 5%, preferably less than 1% and more preferably less than 0.5% water by weight of the mixture.

The composition of the invention can, depending on the active macromolecular principle used therein, be used in the treatment of a variety of conditions and diseases of the human or animal body by therapy or, alternately, can be used to introduce macromolecules essential for the diagnosis of diseases and conditions within the human or animal body. The compositions of the invention are preferably pharmaceutical or cosmetic compositions.

In the composition of the invention, the mixture contained in the capsule may be a liquid, semi-solid or gel, which is either in the form of a solution or a microparticulate dispersion. That is to say the active macromolecular principle(s) for absorption are incorporated into the formulation either in the form of a solution or as a microparticulate dispersion. Alternatively, the composition may be in the form of a solid.

The compositions of the invention are suitably produced by preparing a substantially anhydrous mixture of the active macromolecular principle and the bile salt/additive absorption enhancer and then filling uncoated capsules with the mixture and then coating them with an appropriate polymer mixture to achieve the desired permeability properties. Depending on the nature of additional excipients employed, the pharmaceutical composition of the invention may be in liquid, solid, semi-solid or gel form. The pharmaceutical composition of the invention is suitable for administration via any route giving access to different mucosal tissues such as buccal and sublingual mucosa, the nasal palate, the lungs, the rectum, the intestinal tract (including the large and small intestines) and the vagina. In the case of liquid, semi-solid or gel formulations, these may be either anhydrous or aqueous.

Where the intended site of action of the composition of the invention is the intestine, it is desirable that the composition is enclosed within an enteric coating which can withstand the stomach, so that the components of the formulation remain together, undiluted and in close association until they reach the tissues of the small intestine or colon. Such formulations will suitably be anhydrous. Compositions in liquid form will suitably be administered as enteric-coated capsules, while solid formulations may be administered either within enteric-coated capsules, or in tablet form.

The enteric coating is chosen appropriately to withstand the natural condition of the stomach and to become permeable at the desired location in the intestine. This is preferably determined by the pH conditions that modulate along the length of the intestine. Where the site of action is the small intestine, it is preferred that the enteric coating becomes permeable and releases its contents at a pH of from 3 to 7, preferably from 5.5 to 7, more preferably from 5.5 to 6.5. Where the intended site of action is the colon, it is preferred that the enteric coating becomes permeable and releases its contents at a pH of 6.8 or above.

Suitable enteric coatings are well known in the art and include polymethacrylates such as those selected from the L and S series of Eudragits in particular Eudragits L12.5P, L12.5, L100, L100-55, L30 D-55, S12.5P, S12.5 and S100. Selection of an appropriate coating for the capsule, which is preferably a gelatine capsule, can readily be made by the person skilled in the art based on their knowledge and the available literature supporting the Eudragit products.

Where the intended site of action is the nasal mucosa, the formulation may be in the form of an aqueous solution or as a dry powder, which can be administered as a spray.

Where the intended site of action is the rectum, an appropriate method of administration is as an anhydrous liquid or solid enclosed within a capsular shell, or incorporated into the matrix of an erodible suppository.

For vaginal application, adminstration of the formulation in gel form is also appropriate.

In the attached Figures:

Figures 1A and 1B show the solubility of a chenodeoxycholate/propyl gallate mixture (Fig 1A) and chenodeoxycholate alone (Fig 1B) in a simulated intestinal fluid.

Figure 2 shows the solubility of a chenodeoxycholate/caproate mixture in a simulated intestinal fluid.

Figure 3 shows the solubility of a deoxycholate/propyl gallate mixture in a simulated intestinal fluid.

Figure 4 shows the olubility of a cholate/sodium caproate mixture in a simulated intestinal fluid.

The following Examples serve to illustrate the present invention and should not be construed as limiting.

EXAMPLES

Example 1 Preparation of chenodeoxycholate/propyl gallate mixture containing insulin as active macromolecular principle

1g of chenodeoxycholic acid is dissolved in 3.4g of sodium hydroxide solution (30mg/ml) with warming. The solution is brought to room temperature and adjusted to pH 7.85 by addition of further sodium hydroxide solution. 500mg of propyl gallate is then added and dissolved by shaking at room temperature. The pH is adjusted to 7.45, and 56.25mg of recombinant human insulin is added with stirring at 37°C. After all the solid has dissolved, the solution is made up to a total weight of 8g with distilled water, frozen and lyophilised overnight to give a dry crystalline powder.

A formulation containing chenodeoxycholate alone is prepared in an identical manner to that described above, except that propyl gallate is omitted where appropriate. As before, the pH of the final solution before drying is adjusted to lie between 7.4 and 7.5.

Example 2 Preparation of chenodeoxycholate/sodium caproate mixture containing insulin as active macromolecular principle

An identical method to that described in Example 1 is employed, except that sodium caproate is used instead of propyl gallate, and is mixed with the chenodeoxycholic acid prior to dissolution in 5.5ml of sodium hydroxide (15mg/ml).

Example 3 Preparation of chenodeoxycholate/sodium oleate mixture

An identical method to that described in Example 2 is employed, except that sodium oleate is used instead of sodium caproate, and insulin is omitted.

Example 4 Preparation of chenodeoxycholate/sodium laurate mixture

An identical method to that described in Example 3 is employed, except that sodium laurate is used instead of sodium oleate.

Example 5 Preparation of chenodeoxycholate/sodium caprate mixture

An identical method to that described in Example 3 is employed, except that sodium caprate is used instead of sodium oleate.

Example 6 Preparation of chenodeoxycholate/butylated hydroxy anisole mixture

An identical method to that described in Example 3 is employed, except that butylated hydroxy anisole is used instead of sodium oleate.

Example 7 Dissolution of chenodeoxycholate, with or without propyl gallate, in simulated intestinal fluid.

Simulated intestinal fluids are prepared according to the recipes given below, where '-', 'L' and 'H' refers to different concentrations of salt in the fluids – zero, low and high respectively.

Low pH buffer (fed state)				
Component	**pH 5.0/-**	**pH 5.0/L**	**pH 5.0/H**	
Acetic acid	8.65g	8.65g	8.65g	
Sodium taurocholate	8.12g	8.12g	8.12g	15mM
Lecithin	2.8g	2.8g	2.8g	3.75mM
Potassium Chloride	-	7.7g	15.2g	
Distilled water	1 litre	1 litre	1 litre	
Sodium hydroxide	to pH 5	to pH 5	to pH 5	

Low pH buffer (fasted state)				
Component	**pH 6.5/-**	**pH 6.5/L**	**pH 6.5/H**	
Potassium di-hydrogen phosphate	3.9g	3.9g	3.9g	
Sodium taurocholate	1.6g	1.6g	1.6g	3mM
Lecithin	0.6g	0.6g	0.6g	0.75mM
Potassium Chloride	-	7.7g	15.2g	
Distilled water	1 litre	1 litre	1 litre	
Sodium hydroxide	to pH 6.5	to pH 6.5	to pH 6.5	

6 x 10mg of dry powders as prepared in example 1 are placed in separate wells of a flat-bottomed 96-well microtitre plate, and successive aliquots of 25µl of SIF are added at 15 minute intervals with incubation at 37°C. Solubility is assessed after gentle mixing by determining the optical clarity of the well contents in a plate reader

at 620nm. Increasing optical density (>0.5) in the graphs of Figures 1A and1B indicates the presence of precipitates. As can be seen, when 10mg chenodeoxycholate is diluted out to 150µl volume, precipitation occurs under all conditions tested, in contrast to the chenodeoxycholate/propyl gallate mixture, which remains dissolved in all variants of simulated intestinal fluid tested.

Example 8 Dissolution of chenodeoxycholate/sodium caprate mixture in simulated intestinal fluid.

Identical conditions to those employed in Example 7 are used, except that a chenodeoxycholate/caproate mixture as prepared in Example 5 is employed. As can be seen from Figure 2, essentially clear solutions are obtained upon dilution of 10mg in up to 200µl of all intestinal fluid buffers described.

Example 9 Dissolution of chenodeoxycholate/sodium oleate mixture in simulated intestinal fluid.

Identical conditions to those employed in Example 7 are used, except that a chenodeoxycholate/oleate mixture as prepared in Example 3 is employed. Essentially clear solutions are obtained upon dilution of 10mg in up to 200µl of all intestinal fluid buffers described.

Example 10 Dissolution of chenodeoxycholate/sodium laurate mixture in simulated intestinal fluid.

Identical conditions to those employed in Example 7 are used, except that a chenodeoxycholate/laurate mixture as prepared in Example 4 is employed. Essentially clear solutions are obtained upon dilution of 10mg in up to 200µl of all intestinal fluid buffers described.

Example 11 Dissolution of chenodeoxycholate/sodium caproate/insulin mixture in simulated intestinal fluid.

Identical conditions to those employed in Example 7 are used, except that a chenodeoxycholate/caprate mixture as prepared in Example 2 is employed.

Essentially clear solutions are obtained upon dilution of 10mg in up to 200µl of all intestinal fluid buffers described.

Example 12 Dissolution of chenodeoxycholate/butylated hydroxy anisole mixture in simulated intestinal fluid.

Identical conditions to those employed in Example 7 are used, except that a chenodeoxycholate/butylated hydroxy anisole mixture as prepared in Example 6 is employed. Essentially clear solutions are obtained upon dilution of 10mg in up to 200µl of all intestinal fluid buffers described.

Example 13 Dissolution of deoxycholate/propyl gallate mixture in simulated intestinal fluid.

Identical conditions to those employed in Example 7 are used, except that deoxycholic acid is employed instead of chenodeoxycholic acid, and the ratio of bile salt to propyl gallate is 1:1 wt:wt. Essentially clear solutions are obtained upon dilution of 10mg in up to 200µl of intestinal fluid buffer at pH 6.5, in contrast to formulations where sodium deoxycholate alone is employed. The results are shown in Figure 3.

Example 14 In vivo efficacy of chenodeoxycholate/propyl gallate/insulin mixture in juvenile pigs.

Formulations as prepared in example 1 (containing 100iu insulin, 66mg chenodeoxycholic acid and 33mg propyl gallate or 100iu insulin and chenodeoxycholic acid alone) are administered as dry compacted powders via a stoma into the jejunum of eight juvenile pigs (each ~40kg weight). Blood glucose levels are measured at intervals over a six hour period and mean change in AUC of plasma glucose is calculated in h.mmol/l. As can be seen from the summary of data below, inclusion of PG in the formulation gives a higher degree of reproducibility, and a higher degree of efficacy.

	Number of responders	AUC
Insulin/Cheno/PG	8/8	-3.23
Insulin/Cheno alone	4/8	-1.40

Example 15 Dissolution of cholate/sodium caproate mixture in simulated intestinal fluid.

Identical conditions to those employed in Example 7 are used, except that cholic acid is employed instead of chenodeoxycholic acid, and sodium caprylate is employed instead of propyl gallate. Essentially clear solutions are obtained upon dilution of 10mg in up to 200µl of intestinal fluid buffer at pH 5, in contrast to formulations where sodium cholate alone is employed. The results are shown in Figure 4.

CLAIMS

1. A pharmaceutical composition comprising a mixture of :

(a) an active macromolecular principle; and

(b) a non-conjugated bile acid or salt; and

(c) an additive chosen from propyl gallate, butyl hydroxy anisole (BHA) and analogues and derivatives thereof, and an organic acid or a pharmaceutically acceptable salt thereof, or mixtures thereof, which additive is capable of allowing the non-conjugated bile salt to remain in solution when added to intestinal fluids at pH levels between 5 and 6.5, and which, when introduced into the intestine does not raise the pH of the intestinal fluid above pH 7.5.

2. A composition according to claim 1, which comprises less than 5% by weight of water.

3. A composition according to any of claims 1 to 2, wherein the composition is coated with an enteric coating which becomes permeable at a pH from 3 to 7.

4. A composition according to any one of claims 1 to 3, wherein the mixture comprises at least 1% by weight of the additive (c).

5. A composition according to any one of claims 1 to 4, wherein the ratio by weight of the non-conjugated bile salt/additive (b + c) to active macromolecular principle is at least 5:1.

6. A composition according to any preceding claim, wherein the mixture is in the form of a solution or a microparticulate dispersion.

7. A composition according to any preceding claim, wherein the mixture is in solid form.

8. A composition according to any one of claims 1 to 7, wherein the active macromolecular principle is a polypeptide or protein, polynucleotide, polysaccharide or a mixture thereof.

9. A composition according to claim 8, where the active macromolecular principle is chosen form insulin, calcitonin, growth hormone, parathyroid hormone, or erythropoeitin, and derivatives and analogues thereof, either synthetic or from natural sources, conforming to structures derived from either human or animal origin.

10. A composition according to claim 9, where the active macromolecular principle is insulin or a derivative or analogue thereof, either synthetic or from natural sources, conforming to structures derived from either human or animal origin, and the composition further comprises an insulin sensitising agent.

11. A composition according to any preceding claim, wherein the non-conjugated bile acid or salt is chenodeoxycholate.

12. A composition according to any preceding claim, wherein the additive is chosen from propyl gallate or an analogue or a derivative thereof, including esters of gallic acid, where the esters may be linear or branched chain C_{1-12} alkyl, C_{1-12} alkyloxy, C_{1-12} alkylthio or C_{2-12} alkenyl esters, and the compounds are optionally substituted with halogen, linear or branched chain C_{1-12} alkyl, C_{1-12} alkyloxy, C_{1-12} alkylthio or C_{2-12} alkenyl esters.

13. A composition according to any of claims 1 to 11, wherein the additive is chosen from BHA or an analogue or derivative thereof, including analogues and derivatives of hydroxy anisole where the methyl group or the methoxy group linked to the aromatic ring and/or the hydrogen ortho to the hydroxyl group are replaced by linear or branched chain C_{1-12} alkyl, C_{1-12} alkyloxy, C_{1-12} alkylthio or C_{2-12} alkenyl, either unsubstituted or substituted in any position, especially by halogen atoms.

14. A composition according to any preceding claim, for use in the therapeutic or diagnostic treatment of the human or animal body.

15. Use, in a pharmaceutical composition, of a non-conjugated bile acid or salt, together with an additive chosen from propyl gallate and BHA and analogues and derivatives thereof, and an organic acid or a pharmaceutically acceptable salt thereof, or mixtures thereof as an enhancer for the absorption of macromolecules across the intestinal wall.

16. Use of a non-conjugated bile acid or salt , together with an additive chosen from propyl gallate and BHA and analogues and derivatives thereof, and an organic acid or a pharmaceutically acceptable salt thereof, or mixtures thereof in the manufacture of a medicament containing an active macromolecular principle, in order to enhance absorption of the active macromolecular principle into the human or animal body.

17. Use according to claims 15 or 16, wherein the molecule(s)/active macromolecular principle to be absorbed is a polypeptide or protein, polynucleotide, polysaccharide or a mixture thereof.

18. Use according to claim 17, wherein the molecule(s)/active macromolecular principle to be absorbed is chosen form insulin, calcitonin, growth hormone, parathyroid hormone, or erythropoeitin, and derivatives and analogues thereof, either synthetic or from natural sources, conforming to structures derived from either human or animal origin.

19. Use according to claim 18, wherein the molecule(s)/active macromolecular principle to be absorbed is insulin or a derivatives or analogue thereof, either synthetic or from natural sources, conforming to structures derived from either human or animal origin, and the composition or medicament further comprises an insulin sensitising agent.

20. Use according to any one of claims 15 to 19, wherein the composition comprises less than 5% by weight of water.

21. Use according to claims 15 to 19, which comprises incorporating the active macromolecular principle(s) to be absorbed into the aromatic alcohol in the form of a solution, as a microparticulate dispersion or as a solid.

22. A method of enhancing the absorption of an active macromolecular principle in a patient, which method comprises administering to said patient a composition as defined in any one of claims 1 to 14.

23. A method of treating a patient suffering from a condition or disease treatable by administration of a composition according to any of claims 1 to 14.

ABSTRACT

The invention provides a pharmaceutical composition comprising a mixture of :

(a) an active macromolecular principle; and

(b) a non-conjugated bile acid or salt; and

(c) an additive chosen from propyl gallate, butyl hydroxy anisole (BHA) and analogues and derivatives thereof, and an organic acid or a pharmaceutically acceptable salt thereof, or mixtures thereof, which additive is capable of allowing the non-conjugated bile salt to remain in solution when added to intestinal fluids at pH levels between 5 and 6.5, and which, when introduced into the intestine does not raise the pH of the intestinal fluid above pH 7.5.

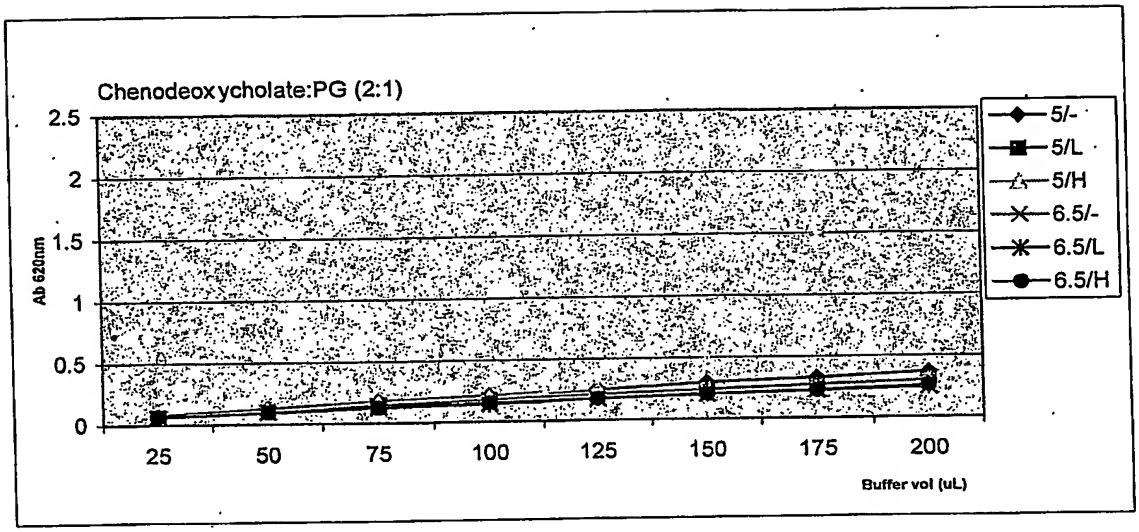

FIGURE 1A

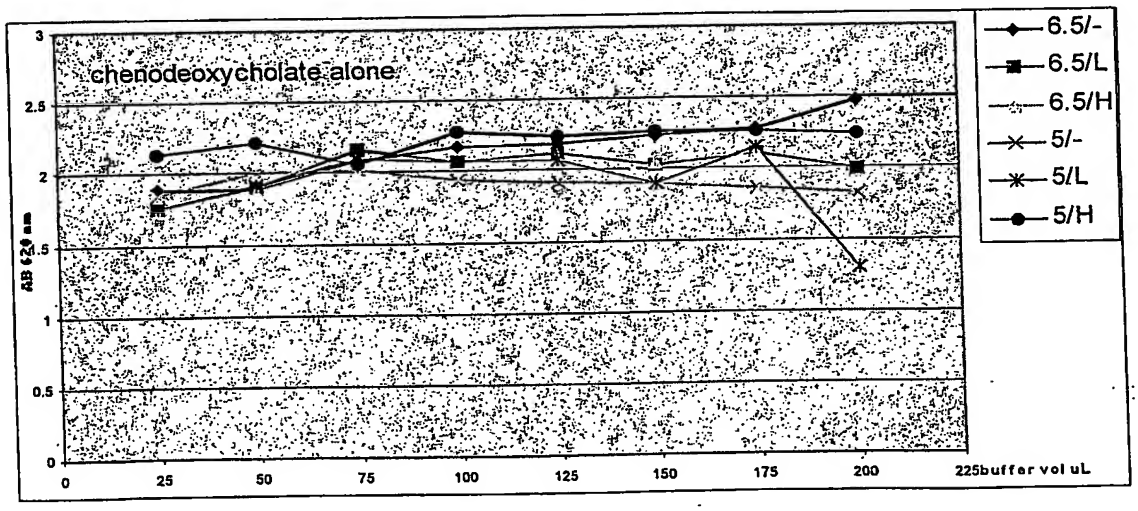

FIGURE 1B

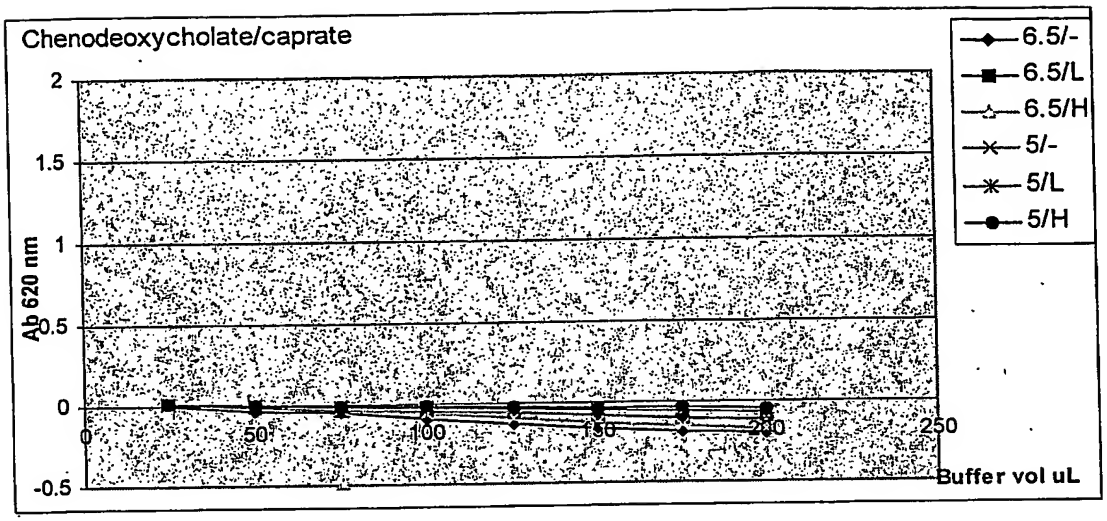

FIGURE 2

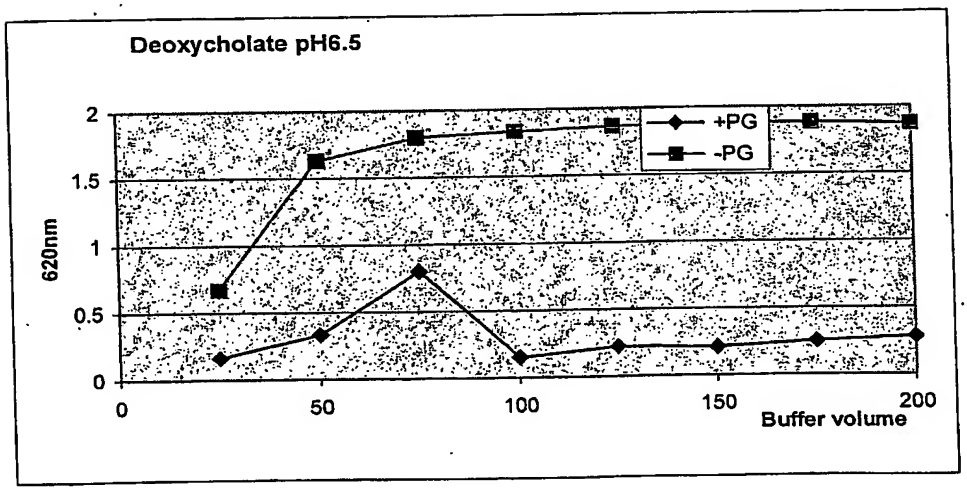

FIGURE 3

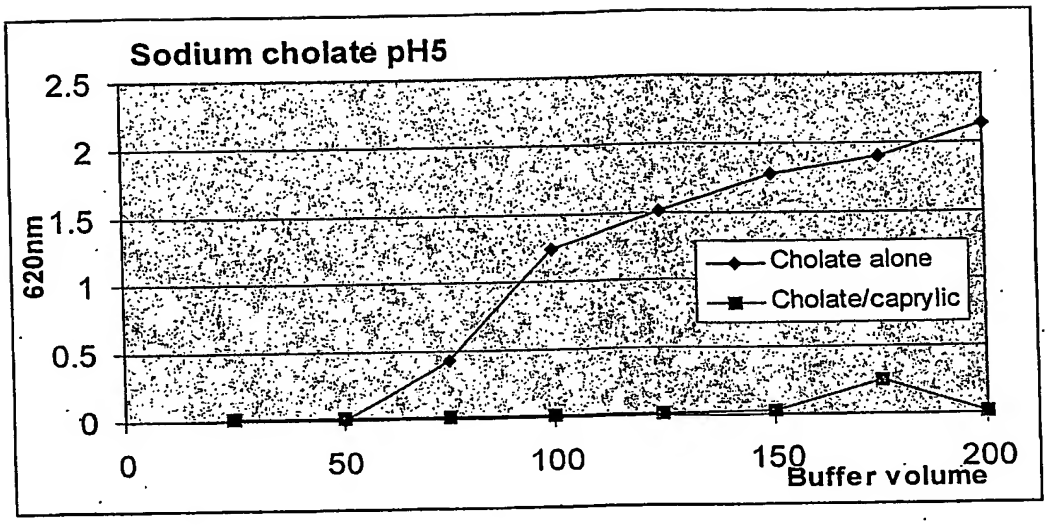
Sodium cholate pH5
2.5
2
1.5
1
0.5
0
620nm
0
50
100
150
200
Buffer volume
Cholate alone
Cholate/caprylic

FIGURE 4